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The pharmacological profile of δ opioid receptor ligands, (+) and (−) TAN-67 on pain modulation

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Abstract

We designed the nonpeptidic highly selective δ opioid receptor agonist on the basis of message address concept and the accessory site theory and synthesized (±) TAN-67. In spite of highly potent agonistic activity in *in vitro* assay, (±) TAN-67 (racemate) afforded a weak antinociceptive effect in the mouse tail-flick test. This result led us to separate (±) TAN-67 to optical pure compounds, (+) and (−) TAN-67. An i.t.-treatment with (−) TAN-67 produced profound antinociceptive effects through specifically acting on δ_1 receptors. Unlike (−) TAN-67, i.t.-administered (+) TAN-67 displayed dose-related nociceptive behaviors such as scratching, biting and licking. The effect of (+) TAN-67 was blocked by i.t.-treatment with NTI (δ receptor antagonist) and (−) TAN-67 (δ_1 receptor agonist), but not by morphine (μ receptor agonist). The mechanisms involved in spinal pain modulation induced by (+) and (−) TAN-67 were also described.

Keywords: δ Opioid receptor; Antinociception; Hyperalgesia

Introduction

Opioid receptors were generally classified to three types (μ, δ, κ) not only by pharmacological studies but also by molecular biological studies [1, 2]. Although many highly selective and potent δ opioid receptor agonists are now available for studies on this receptor, almost all of them are peptide such as DPDPE [2]. Recently, nonpeptidic δ opioid receptor agonists BW373U86 [2], SNC80 [2], SIOM [2], and SB 219825 [3] have been reported, but more selective (including subtype selectivity) and potent agonists are still needed to investigate the properties of the δ opioid receptor. We designed highly selective and potent δ opioid receptor agonist on the basis of message address concept [4] and the accessory site theory

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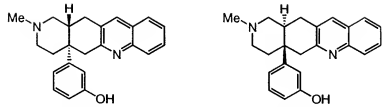

Fig. 1. The structure of (−) and (+) TAN-67.

[5] and synthesized (4a*S* *,12a*R* *)-4a-(3-hydroxyphenyl)-2-methyl-1,2,3,4,4a,5,12,12a-octahydropyrido[3,4-*b*]acridine, (±) TAN-67 [6]. Then, we separated (±) TAN-67 to optical pure compounds, (+) and (−) TAN-67, and investigated the pharmacological profiles of these ligands in detail (fig. 1). Herein, we report the rational drug design of (±) TAN-67 and the pharmacological profiles of (±), (+), and (−) TAN-67.

Rational drug design

In the presumed model for the binding of selective nonpeptidic δ opioid antagonist, NTI [2], with δ opioid receptor, we considered the three pharmacophore binding sites at the morphinan moiety (message part): an ionic interaction with the cationic part of NTI (protonated 17-nitrogen), a π-π interaction with the aromatic moiety, and a hydrogen bond with the 3-hydroxy group, and the one δ opioid receptor specific interactions at the address part: a π-π interaction of the indole aromatic ring of NTI to δ receptor (Fig. 2. a)). This binding model for NTI in combination with the accessory site model[5] led us to design novel selective δ opioid receptor agonists. Thus, we assumed that the drastic conformational change of δ opioid receptor would need for agonistic activity and that the conformationally fixed phenol moiety of NTI would not be suitable disposition for elicitation of agonistic activity. This hypothesis was supported by several reports in which the comparison of agonistic activity of morphine having fixed phenol moiety with phentanyl derivatives having rotatable aromatic ring [7] and the comparison of opioid antagonist naltrexone having fixed phenol moiety with opioid agonist trans-4a-aryldecahydroisoquinoline having rotatable aromatic ring [8] were described. Therefor, we designed and synthesized compound **1** (4,5-epoxy and 10-methylene moieties were removed from NTI) which can rotate to a suitable position by induced fit for the agonistic interaction with the receptor (Fig. 2. b)). Contrary to our expectation, compound **1** showed no agonist activity in the mouse vas deferens (MVD) assay. However, after investigating the structure-activity relationship in this series of compounds, we found out that the compound **2** having 7-F-substituent in compound **1** showed opioid δ selective partial agonist activity in MVD assay. Furthermore, none of the compounds, including the 7-Cl, Br, Me, NO_2, and 8-, 9-, or 10-F derivatives of compound **1**, showed agonistic activity. From these observations, we assumed that the only 7-F-substituent could form a hydrogen bond with the specific site of the receptor to lead agonistic activity (Fig. 2. c)). In other words, this hydrogen binding site, which we call a "fifth pharmacophore", should be important for its agonist character. We

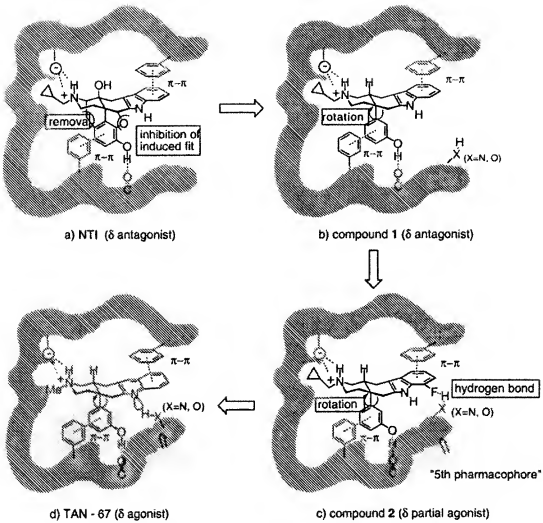

Fig. 2. Possible binding model of each ligand with the δ opioid receptor.

then designed and synthesized compounds which are fused quinoline to decahydroisoquinoline skeleton, because it was expected that the lone pair electron on a nitrogen atom in the quinoline ring could form a hydrogen bond with the receptor more effectively to show more potent agonistic activity than 7-F-indolo derivative **2**. Finally, we found that (±) TAN-67, designed as mentioned above, was highly selective and potent δ opioid receptor agonist. The presumed model for the binding of TAN-67 with the δ opioid receptor is shown in Fig. 2. d).

Pharmacology of (±) TAN-67

(±) TAN-67 showed high selectivity for the δ opioid receptor (Ki=1.12 nM) in guinea-pig cerebrum with 2070-fold higher affinity against μ opioid receptor and 1600-fold higher

affinity against κ opioid receptor. Additionally, it was a potent δ opioid agonist (IC_{50} = 6.61 nM) in the MVD assay that was reversed by NTI (Ke=0.21) [6]. In spite of highly potent agonistic activity in *in vitro* assay, (±) TAN-67 afforded a weak antinociceptive effect in the mouse tail-flick test [9]. In order to find the cause of its weak antinociceptive effect, we separated (±) TAN-67 to optical pure compounds, (+) and (−) TAN-67, by the optical resolution method by using optically active acid.

Pharmacology of (−) TAN-67

The i.t.-administration of (−)TAN-67, which was the same absolute configuration found in natural (−) morphine, produced profound antinociceptive effects in dose dependent manner (ED_{50} = 17.1 nM/mouse) [8]. The antinociceptive effect was blocked by the i.t.-pretreatment with BNTX (δ_1 selective antagonist), but not by NTB (δ_2 selective antagonist), CTOP (μ selective antagonist), or nor-BNI (κ selective antagonist). This results suggested the antinociceptive effect of (−) TAN-67 would be mediated by the stimulation of δ_1 but not δ_2, μ, or κ opioid receptors [10].

Pharmacology of (+) TAN-67

(+) TAN-67 given i.t. caused pain-like nociceptive behavior. The nociception elicited by (+) TAN-67 was significantly attenuated by a δ opioid receptor antagonist NTI. The (+) TAN-67-induced nociception was also suppressed by a δ_1 opioid receptor agonist (−) TAN-67. On the contrary, a prototypical μ opioid receptor agonist morphine did not have any effect on the (+) TAN-67-induced nociception. Furthermore, the nociception evoked by i.t. injection of (+) TAN-67 was attenuated by a non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist. The dose-response curve for the (+) TAN-67-induced nociception was shifted to the right by a selective protein kinase C (PKC) inhibitor. These findings indicate that, like neuropathic pain, a single i.t. injection of (+) TAN-67 appears to induce the sensitization of NMDA receptors associated with PKC. Thus, it is likely that the nociception caused by (+) TAN-67 given i.t. could be a useful pharmacological model for elucidation of the pain mechanisms in the spinal cord.

Conclusion

We designed nonpeptidic highly selective and potent δ opioid receptor agonist on the basis of message address concept and the accessory site theory, and synthesized (±), (−), and (+) TAN-67. As a result of the investigation of their pharmacological profiles, (−) TAN-67 i.t.-administered showed more potent analgesic effect than its racemate (±) TAN-67. Contrary to (−) TAN-67, (+) TAN-67 i.t.-administered displayed dose-related nociceptive behaviors such as scratching, biting and licking. The effect of (+) TAN-67 was blocked by both a non-competitive NMDA receptor antagonist and a selective PKC inhibitor. These finding suggests that the (+) TAN-67-induced nociception resembles neuropathic pain. Thus, (−) and (+) TAN-67 could be useful tools in order to investigate the mechanisms involved in spinal pain modulation.

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